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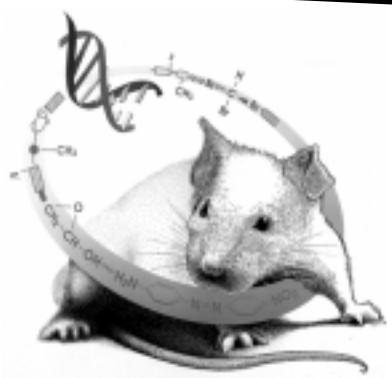

The p53 Workgroup Newsletter

The p53 Workgroup Newsletter is published by the p53 Assay Working Group (AWG) as a means of communication for the HESI's Alternative to Carcinogenicity Testing Committee (ACT).

Letter and article submissions are welcome. Persons interested in contributing to the newsletter should contact:

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On the Inside:

Welcome to the First Issue of the p53 Workgroup Newsletter

by R. Storer, Merck & Co., Inc.

In response to popular demand, the p53 Assay Working Group (AWG) is following the lead established by the TG.AC AWG in publishing a newsletter for the ILSI/HESI Alternatives to Carcinogenicity Testing Technical Committee. We would like to express our thanks to the TG.AC AWG, and especially Sylvia Furst at Boehringer Ingelheim Pharmaceuticals, Inc. for sharing their newsletter template with us so that we, as novice desktop publishers, could get off to a fast start! In order to make the newsletter timely and informative, we need your help!! Please submit news items, articles, ideas for forthcoming issues (Bi-monthly, if possible).

Overview of p53 AWG Pathology Review Committee

by G. Long, Lilly Research Laboratories

The sponsor of studies conducted as part of the ILSI/HESI ACT project is responsible for conducting pathology review of all proliferative lesions, utilizing standard diagnostic criteria (i.e., Standardized nomenclature of proliferative lesions, Society of Toxicologic Pathologists and other standard references). Some lesions in p53 hemizygous mice may be unusual and/or not fit into accepted standardized diagnostic criteria. In these cases, the sponsor pathologist should confer with the $p53^{+/-}$ Assay Working Group (AWG) pathologist, Dr. Gerald Long. The AWG pathologist will confer with the HESI ACT Pathology Subcommittee to decide on the approach to be taken. In most cases, proliferative lesions with problematic diagnoses will be reviewed by a group of pathologists to arrive at consensus diagnoses. For the $p53^{+/-}$ model, this group consists of Gerald Long (contact), John Sagartz, Eugenia Floyd, Joel Mahler, and Charles Montgomery. The sponsor will be asked to provide a set of slides to the contact pathologist who will route them to the members of the review group and receive input to determine consensus opinions. If a consensus can not be reached through this routing, the contact pathologist will, with advice of the Pathology Subcommittee and the sponsor, determine what other action (i.e., a group meeting), will be taken to arrive at a consensus. Once consensus diagnoses are determined, they will be sent back to the sponsor for inclusion in the final report.

For more information, contact Gerald Long at (317) 277-4711.

Phenobarbital Does Not Promote Hepatic Tumorigenesis in a 26-Week Bioassay in *p53* Heterozygous Mice

John E. Sagartz, Sandra W. Curtiss, Roderick T. Bunch, Julio C. Davila, Dale L. Morris, and Carl L. Alden
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The international pharmaceutical community now recognizes genetically-altered mouse bioassays as alternatives to the lifetime mouse cancer study in the safety assessment of pharmaceuticals. The majority of rodent responses to pharmaceutical agents are not considered predictive for humans. A genetically-altered mouse model that is negative for rodent carcinogens which are also human non-carcinogens would certainly be an improvement on traditional testing. The *p53* heterozygous mouse model conceptually represents an intriguing opportunity to improve the cancer hazard identification process. *p53* is the most frequently altered tumor suppressor gene in human cancers, and is an important negative regulator of cell growth and gene expression. Experimentally, the *p53* heterozygous mouse model is showing good concordance with genotoxic rodent carcinogens. We have tested this model with the prototype liver tumor promoter phenobarbital as part of the ILSI HESI consortium activity. Phenobarbital is generally recognized as a non-genotoxic agent which is a potent liver carcinogen in mice, potent liver tumor promoter in rats, and a non-carcinogen in humans.

The tumorigenic potential of phenobarbital was examined in a twenty-six week carcinogenesis bioassay using *p53* heterozygous mice and wild-type controls. 15 mice per sex per genotype were exposed to either 500 or 1000 ppm phenobarbital in the diet. Dietary administration of 3750 ppm *p*-cresidine, a trans-species mutagenic carcinogen, to both heterozygous and wild-type mice served as a positive control.

Phenobarbital. There were no tumors in mice of either sex or genotype treated with phenobarbital. The livers of all animals of both sexes and both genotypes exhibited moderate to marked centrilobular hepatocellular hypertrophy, a finding consistent with an observed increase in absolute and relative liver weights and interpreted as secondary to robust metabolic enzyme induction.

p-Cresidine. Treatment-related histopathology findings were observed in the lower urinary tract, liver, and kidney of *p*-cresidine-treated mice. A progression of proliferative lesions from diffuse transitional epithelial hyperplasia, predominantly sessile and exophytic, to focal dysplasia was observed. Squamous metaplasia of the transitional epithelium was present in mice of both sexes and genotypes. Transitional cell carcinomas were observed in the urinary bladder of 10/14 (71%) heterozygous males and 11/15 (73%) heterozygous females. An additional heterozygous male had a urethral transitional cell carcinoma in the prostatic urethra. One/15 (7%) wild-type males and 1/15 (7%) wild-type females also had transitional cell carcinomas of the urinary bladder histologically identical to those observed in heterozygotes. One heterozygous male mouse treated with *p*-cresidine had a thymic lymphosarcoma with infiltration into the intercostal musculature. The singular incidence of this finding coupled with an emerging recognition of lymphosarcoma as a common spontaneous neoplasm in *p53* heterozygous mice mitigates against its relationship to treatment.

The lack of a phenobarbital-related tumorigenic response in the liver of *p53* heterozygous mice supports the continued examination of this model as an alternative to the 2-year mouse bioassay. Phenobarbital, a potent and prototypic hepatic microsomal enzyme inducer, has been well described as a positive tumorigenic agent in the mouse liver and is a rat thyroid and liver tumor promoter. However, epidemiologic data does not support a role for phenobarbital as a human carcinogen. Since the ultimate purpose of carcinogenesis bioas-

says is to predict human cancer risk, the *p53* heterozygous mouse model is attractive in its potential to eliminate false positive responses for human carcinogenic risk with molecules of this class.

The ability of the *p53* heterozygous mouse assay to identify genotoxic agents was again confirmed in this study. Both heterozygous and wild-type mice exhibited a positive urinary bladder tumor response to *p*-cresidine in addition to pre-neoplastic mucosal epithelial hyperplasia, dysplasia, and squamous metaplasia. Previous reports have not identified the potential of *p*-cresidine to induce urinary bladder tumors in wild-type mice within this time frame. In our study, we did not reproduce the weak positive liver tumor response to *p*-cresidine observed in female mice in the 2-year mouse bioassay nor that previously observed in male *p53* heterozygous mice.

Our results demonstrate the lack of a hepatic tumor response to phenobarbital, a compound which is a potent and prototypic hepatic microsomal enzyme inducer, a non-genotoxic rodent carcinogen, and a human non-carcinogen. This finding supports the continued utility of this model as an alternative to the mouse bioassay for human carcinogenic safety assessment of potentially genotoxic carcinogens since it did not produce a false positive response to this potent non-genotoxic agent.

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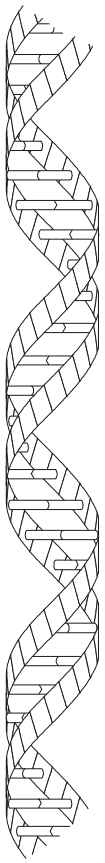
Upcoming Meetings and Events

- ☺ 1st Annual Meeting on Rodent Models in Modern Risk Assessment, September 8-12, 1998, The Jackson Laboratory, Bar Harbor, Maine
- ☺ New Cancer Strategies: p53. September 16-17, 1998, Renaissance Hotel, Washington, D.C. Sponsored by Cambridge Healthtech Institute
- ☺ Cellular Targets of Viral Carcinogenesis, American Association for Cancer Research Scientific Conferences, Marriott's Laguna Cliffs Resort, Dana Point, CA, September 24-28, 1998.
- ☺ PhRMA Preclinical Drug Safety Fall Workshop: Issues Related to the Implementation of ICH Guidance Documents on Carcinogenicity Assessment and a FDA/Industry Dialogue on Special Topics. September 28-29, 1998, Doubletree Hotel, Rockville, MD
- ☺ Anti-Cancer Proteins and Drugs: Structure, Function and Design, A New York Academy of Sciences Conference, November 6-9, 1998, New York City
- ☺ Endogenous Sources of Mutations, American Association for Cancer Research Scientific Conferences, Sanibel Harbour Resort and Spa, Fort Myrers, FL, November 11-15, 1998.
- ☺ Symposium VIII– Transgenic Mouse Carcinogenesis Bioassay Models. American College of Toxicology 19th Annual Meeting, November 8-11, 1998, Grosvenor Resort, Orlando, FL
- ☺ Cancer Biology and the Mutant Mouse: New Methods, New Models, New Insights. American Association for Cancer Research Scientific Conferences, Keystone , Colorado, January 31-February 5, 1999.



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In vivo transgenic bioassays and assessment of the carcinogenic potential of pharmaceuticals.
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Recent Results from Testing of Several Genotoxic Carcinogens in p53 Knockout (+/-) Mice in Japan

by Kunitoshi Mitsumori, NIHS, Japan

Heterozygous p53 knockout (p53 +/-) mice are also available in Japan, since Oriental Yeast Co. Ltd. (Tokyo, Japan) is maintaining CBA/OYC - p53 (+/-) mice as an attempt for the validation of the model of short-term carcinogenicity tests. The Division of Pathology at National Institute of Health Sciences (NIHS), Tokyo, Japan, is investigating the carcinogenic potential of N-ethyl-N-nitrosourea (ENU), dimethyl nitrosamine (DMN) and ethyl carbamate (urethane) using p53 (+/-) mice as part of the research on the usefulness of rasH2 and p53 knockout mice for short-term carcinogenicity tests which have been recommended by the ICH.

Twenty female CBA/OYC - p53 (+/-) mice (p53KO mice), 9 weeks old, were assigned to the ENU-treated group and 10 females to the vehicle (saline) control group. Twenty CBA/OYC wild type female littermates (wild type mice) were assigned to the ENU-treated group, and 10 wild type female mice received the vehicle. The mice received one dose of 120 mg/kg ENU or vehicle by a single intraperitoneal injection, and were monitored for 26 weeks without any further treatment. Uterine endometrial sarcomas were induced in both ENU-treated p53KO and wild type mice. The incidence (90%) was significantly increased in the treated p53KO mice, as compared with that in the treated wild type mice (20%). Lung tumors were also induced with high incidences in the treated p53KO and wild type mice, but there was no difference in the incidence between these mice. Thymic lymphomas and subcutaneous sarcomas were induced only in the treated p53KO mice with a low incidence.

Thirty female p53KO mice of 9 weeks old and thirty female wild type littermates received a single intraperitoneal injection of 5 mg/kg DMN. After one week, they were subdivided into two groups consisting of fifteen p53KO and fifteen wild type mice each, and were fed diet containing 500 or 0 ppm of phenobarbital (PB) for 26 weeks. Altered hepatocellular foci were observed in 2 of 10 DMN-treated p53KO mice, but not in DMN-treated wild type mice. In DMN+PB groups, the incidence of altered hepatocellular foci in p53KO mice was significantly increased as compared with wild type mice. In addition, hepatocellular adenomas were induced in 6 of 10 (60%) p53KO mice treated with DMN+PB, while the incidence in wild type mice treated with DMN+PB was 2 of 11 (20%).

Ten male p53KO mice of 9 weeks old and ten female wild type littermates received a single intraperitoneal injection of urethane and were monitored for 20 weeks without any treatment. Lung adenomas were induced both in the treated p53KO and wild type mice, but no differences in the incidence (60% in wild type mice and 80% in p53KO mice) and the multiplicity was observed between them. Further studies using more p53KO mice are necessary to clarify their sensitivity to urethane.

The results of our studies done so far may suggest that p53KO mice derived from the Oriental Yeast are generally sensitive to genotoxic carcinogens as compared with the wild type littermates. However, it is not known whether the tumor sensitivity in the p53KO mice derived from Oriental Yeast is similar to that in the p53KO mice derived from Taconic Farms. Additional studies using other carcinogens are now in progress in our laboratory.

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Experience to Date with the Positive Control Compounds p-Cresidine and Benzene

By R. Storer, Merck and Co. Inc.

Many groups that are in the process of setting up for their 26-week study in $p53^{+/-}$ mice have expressed interest in the results to date with the positive control compounds p-cresidine and benzene. Several groups who have finished their studies have provided data with which we can draw some tentative conclusions. First, p-cresidine appears to be working reliably as a positive control compound for induction of bladder tumors with dietary administration at dose levels from 2500 to 5000 ppm producing higher incidences of lesions than produced by daily gavage dosing in corn oil at 400 mg/kg. Secondly, results for oral administration of 100 mg/kg benzene in corn oil 5 days per week have produced results that are not as encouraging in terms of this compound serving as a reproducible, clear-cut positive control for a 26-week study in this model.

Results for Benzene

Gerry Long at Eli Lilly reports borderline positive results for their benzene positive control group. There were gross lesions suggestive of neoplasias in 3/15 males and 1/15 females. The final diagnoses showed 3/15 males and 1 of 15 females with lymphosarcomas and 1/15 males with an osteosarcoma. For a separate study recently terminated at Huntingdon Life Sciences, a preliminary report from Peter Lyle informed us that there were no gross lesions indicative of neoplasia in the benzene-treated mice. Both of these studies used a dose of 100 mg/kg in corn oil 5 days per week. David Jacobsen-Kram at MA BioServices reports better results in 2 studies in his shop with benzene (200 mg/kg in corn oil, 7 days/week) as the positive control (more details hopefully in the next issue).

Results for p-Cresidine:

Tennant *et al* (1995, 1996) have published the results of the initial short-term bioassays of p-cresidine in $p53^{+/-}$ mice . Their data showed that males are more sensitive than females and that the incidence of bladder transitional cell or squamous cell carcinomas in males ranged from ~87% to 100% in 3 separate bioassays at the high dose of 0.5% in the diet for 24 weeks. John Sagartz also reports good results with p-cresidine in the diet (see article on page 2). Since then, there have been at least 4 studies which have come to necropsy using a 400 mg/kg/day (mpk) gavage dose of p-cresidine in corn oil. In the first of these, Boehringer Ingelheim (BIPI) conducted a pilot study to determine an appropriate positive control dose for p-cresidine using oral gavage administration in corn oil. The data from the BIPI study are shown in Figure 1 below and were used to establish 400 mpk as the recommended high dose for p-cresidine positive control groups for studies in the $p53^{+/-}$ model. A significant decrease in body weight gain was observed over the course of the study.

Figure 1. Incidence of p-Cresidine-Induced Bladder Lesions in a 26-Week Study Conducted at BIPI (Data provided by Dr. Kerry Blanchard)

	Control		p-Cresidine (200 mpk)		p-Cresidine (400 mpk)	
	M	F	M	F	M	F
Neoplasms*	0/6	0/7	3/15	0/15	10/14	4/14
Metaplasia (squamous)	0/6	0/7	3/15	0/15	8/14	7/14
Dysplasia (epithelial)	0/6	0/7	6/15	2/15	7/14	5/14
Hyperplasia (urothelial)	0/6	0/7	15/15	7/15	12/14	8/14
Increased Mitotic Figures	0/6	1/7	15/15	13/15	14/14	13/14

In three subsequent studies with the ILSI ACT protocol with the 400 mpk in corn oil p-cresidine gavage positive control, two produced evidence of tumorigenesis in the urogenital system based on the appearance of gross lesions at necropsy (Merck/clofibrate, Merck/phenacetin; preliminary data) but the microscopic findings are not yet available. In a third study (Bayer/ampicillin), there were no gross lesions observed at terminal necropsy but the following findings were recorded based on microscopic examination (see Figure 2).

Figure 2. Incidence of Bladder Lesions in the p-Cresidine Positive Control Group in a 26-Week Study of Ampicillin (Data provided by Dr. Matthias Rinke, Bayer AG)

	Control		Ampicillin (3000 mpk)		p-Cresidine (400 mpk)	
	M	F	M	F	M	F
Transitional Cell Carcinoma	0/15	0/15	0/15	0/15	6/15	0/14
Transitional Cell Papilloma	0/15	0/15	0/15	0/15	2/15	0/14
Nodular Hyperplasia	0/15	0/15	0/15	0/15	0/15	4/14
Diffuse Simple Hyperplasia	0/15	0/15	0/15	0/15	13/15	13/14
Squamous Metaplasia	0/15	0/15	0/15	0/15	2/15	1/14
Granulocytic Infiltrates	0/15	0/15	0/15	0/15	5/15	2/14
Mast Cell Accumulation	0/15	0/15	1/15	0/15	13/15	7/14

Dr. Rinke observed that, “After treatment with p-cresidine there were obvious sex differences in our study, since no tumors were found in females. The carcinomas of males were mainly very small and it was often arbitrary to differentiate them from hyperplastic lesions that in most cases occurred concurrently. Thus, the diagnoses were mainly made on cellular criteria like mitotic activity and invasive growth. Also with respect to the simple diffuse hyperplasia the average grade of the lesion was higher in males (2.6 = slight to moderate) when compared to females (1.9 = slight). Non-proliferative lesions were slight granulocytic infiltration of the (sub-)mucosa and minimal to slight accumulation of mast cells in the bladder wall”.

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